5-22-2017

Isolation and Comparative Genomic Analysis of Final Third of Satis Genome

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Abstract

A highly novel Streptomyces phage, Satis, was isolated from a direct environmental sample collected from outside Danforth House on the Washington University campus. Satis infects bacterial species *Streptomyces lividans* producing pinpoint, cloudy plaques less than 1mm in diameter. Electron microscope data shows rare atypical physical features. Rather than the common octahedral capsid shape, Satis has a prolate head with visible cross-linked hexagonal protein structure and average measurements of 285 nm by 47 nm with a long, flexible tail measuring 268 nm. Upon sequencing, it was found that Satis contains the longest phage genome discovered to date through the SEA-PHAGE program at 186,702 base pairs. The genome is quite novel in sequence, as its closest genetic match, bacteriophage Chymera, is similar across only 0.2% of the genome. This means that Satis belongs to no known previously characterized cluster and is considered a Singleton phage. The genome contains 325 protein coding genes, of which our group analyzed Gene 230 to the end of the genome. The vast majority of the genes in this section run 3' to 5' and compared to the other two sections, these genes seem to be the most unique in primary, secondary, and tertiary structure. Due to the novelty of Satis, functional evidence from comparative genomic analysis is sparse. We are currently in the process of a more thurough comparative genomic analysis between Satis and other Streptomyces phages, particularly phage JustBecause, another Streptomyces phage isolated by Washington University in St. Louis students in 2016 with similar morphology to Satis.

Isolation and Purification

Figure 1: Isolation site of Phage Satis, outside Danforth House on the campus of Washington University in St. Louis

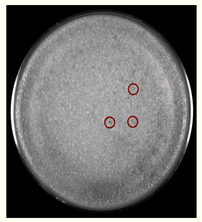


Figure 2: Plate photo showing plaque morphology. Satis creates pinpoint cloudy plaques less than 1mm in diameter as shown by dark spots circled on plate.

Characterization

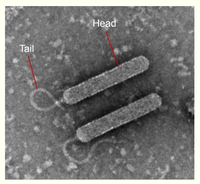


Figure 3

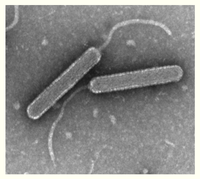


Figure 4

Tail Length	Head Length	Head Width
268 ± 3.8 nm	285 ± 5.3 nm	47 ± 2.1 nm

Table 1: Average size of Satis with standard deviation. Calculated using a sample of five TEM photos and analyzed using ImageJ.

Figure 3: TEM photo of two Satis phages showing prolate head and long flexible tail characteristic of the siphoviridae family of phage. Taken at 25000x magnification.

Figure 4: TEM photo of JustBecause at same magnification, showing very similar size and morphology between the two phage.

Figure 5: Close up of Satis head shell shows interlocking grid of hexagonal capsid proteins.

Figure 5

Annotation of Genes 231-325

Figure 6: The phamerator map for genes 231-325 is shown above. The majority of the genes in this section are orphams meaning they don't fit into any currently annotated protein families in the SEA-PHAGE program.

Gene	Start	Stop	Function
243	145055	144546	Phosphatase Domain of Polynucleotide Kinase
266	153436	152981	Antitoxin DarG

Table 2: Functional annotation calls of final third of Satis genome. Shows highly variable region of genome

Functional Evidence

Gene 243- Phosphatase Domain

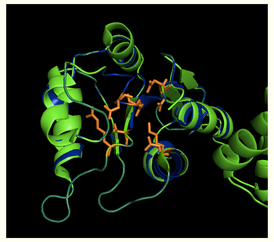


Figure 7: Satis gene 242 (blue) overlaid with the phosphatase domain of T4 phage polynucleotide kinase. Phyre Protein Modeling showed 7 of 8 conserved active sites between the two proteins shown in orange

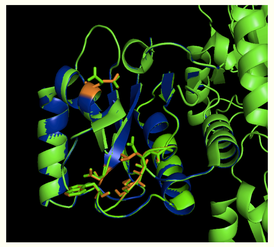


Figure 8: Satis gene 266 (blue) overlaid with DarG antitoxin from *M. tuberculosis*. Phyre Protein Modeling showed 6 out of 9 conserved active sites between the two proteins shown in orange

Acknowledgements & References

We would like to thank our mentors Drs. Christopher Shaffer and Kathleen Hafer, our TA's Ryan Smith, Kendra Woodruff, and Emily Moore, the Washington University in St. Louis Biology Department, the Hatfull Lab, and the Howard Hughes Medical Institute for supporting our research.

Abedon, Stephen T. "Phage Evolution and Ecology." *Advances in Applied Microbiology*, vol. 67, Elsevier, 2009, pp. 1–45.

Software Used: DNAMaster, Glimmer, GeneMarkA, Phyre2, NCBI BLAST, PhagesDB BLAST, HHPRED, PECAAN, PyMol, Starterator, SplitsTree, GenBank, EMBOSS Stretcher

Phage Comparison

Satis vs Chymera

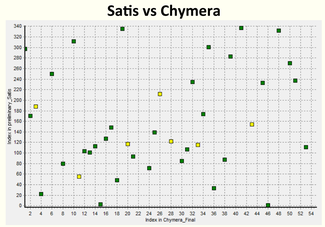


Figure 9: Synteny map of Satis and Streptomyces phage Chymera shows very low level of gene order conservation. Chymera is the closest match to Satis currently published.

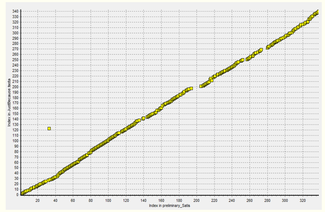


Figure 10: Synteny map of Satis and Streptomyces phage JustBecause also found this year showing extremely high gene order conservation between the two phage.

	Satis	JustBecause	Chymera
Gene Count	325	340	55
tRNA Count	13	0	0
Genome Length	186702 bp	184281 bp	34742 bp

	Satis vs JustBecause	Satis vs Chymera
Gene Order Conservation	0.9774	0.0263
ANI	0.7440	0.5848
Ortholog Number	266	39
Average Ortholog Similarity	0.7467	0.5200
Average Ortholog Identity	0.6450	0.3932
Global Alignment	.639	.159

Table 3 & 4: Comparisons of genomes of JustBecause and Chymera against Satis showing close relation between Satis and JustBecause far exceeding next closest relation

Figure 11 (Bottom): Ortholog map of entire genome of Satis vs JustBecause. Orthologs are shown with same color, unique genes are blacked out

Evolutionary Implications

- Phages display wide genetic diversity: Satis and JustBecause have genomes that are highly unique on both the DNA, amino acid sequence, and protein level
- Multiple mechanisms for phage genomic evolution
  - Satis, part of the siphoviridae family, has a double stranded DNA genome meaning that it can readily recombine with and incorporate bacterial host DNA through transduction (this DNA could be bacterial or phage in origin)
  - Increased gene variety through bacterial vectors; can both incorporate and donate phage DNA to an infecting phage
- Highly conserved gene order (synteny) and high number of orthologs support the existence of a common ancestor for Satis and JustBecause, seen on Splitstree
- Lack of synteny and low number of orthologs with Chymera support existence of a much more distant common ancestor between Satis and its other most closely related *Streptomyces* phage, also shown on Splitstree
- Viruses with higher mutation rates tend to have phenotypes that are less sensitive to mutational change; therefore their gene products are conserved
  - Explains high number of orthologs between Satis and JustBecause
- Possibility: Satis has a higher mutation rate than other Streptomyces phages due to its significantly larger genome
  - Fidelity may be sacrificed for speed of replication to improve Satis' competitive fitness in out-replicating other Streptomyces phages

Ortholog Case Comparisons

Satis 29 v JustBecause 25

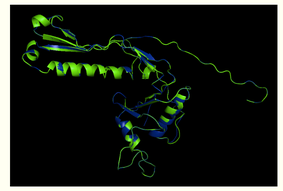


Figure 12 & Table 5: Satis 29 (blue) overlaid with JustBecause 25 using Phyre results left. Table below shows top HHPRED results for both genes. Results show very conserved secondary and tertiary structure.

Table 5	Start Coordinate	Stop Coordinate	Top Result	Description	Probability	Coverage	E-Value
Satis 29	16906	18219	pfam10124	Mu-like prophage major head subunit gpT	100	56.06	6.6e-30
JustBecause 25	15649	16962	1ohg_a	Major capsid protein; Baceriophage HK97	97.75	62.01	6.6e-4

Satis 144 v JustBecause 144

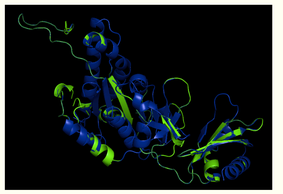
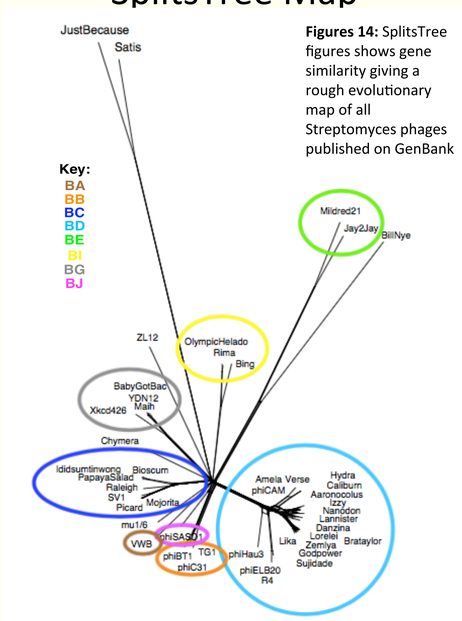


Figure 13 & Table 6: Satis 144 (blue) overlaid with JustBecause 144 using Phyre results left. Table below shows top HHPRED results for both genes. Results show very conserved secondary and tertiary structure.

Table 6	Start Coordinate	Stop Coordinate	Top Result	Description	Probability	Coverage	E-Value
Satis 144	90395	89094	2iut_A	DNA translocase FTSK	100	94.67	0
JustBecause 144	90307	89009	2iut_A	DNA translocase FTSK	100	99.3	4e-39

SplitsTree Map



Figures 14: SplitsTree figures shows gene similarity giving a rough evolutionary map of all Streptomyces phages published on GenBank